PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P37821WO GWS	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2004/004401	International filing date *(day/month/year)* 15.10.2004	Priority date *(day/month/year)* 16.10.2003
International Patent Classification (IPC) or national classification and IPC C12N5/00, C12N5/06, C07K14/475		
Applicant UNIVERSITY OF EDINBURGH et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07.04.2005	28.12.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Nichogiannopoulou, A Telephone No. +49 89 2399-8054

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/004401

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-48 as originally filed

Sequence listings part of the description, Pages

1-6 as originally filed

Claims, Numbers

1-30 received on 13.09.2005 with letter of 12.09.2005

Drawings, Sheets

1/10-10/10 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/004401

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

   see separate sheet

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-30

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☒ the claims, or said claims Nos. 1-30 are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-30, all partially

   ☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

   ☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

   ☒ See separate sheet for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/004401

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☒ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-29
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-29
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-22, 28-30
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I

Basis of the report

1. The amendments filed with the letter of 12.09.2005 are formally allowable under Article 34(2)(b) PCT because they do not introduce subject-matter extending beyond the content of the application as filed.

Re Item II

Priority

1.1. The following documents were published prior to the international filing date but later than the priority date claimed (P-documents):

P1: YING QI-LONG ET AL: "BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3." CELL, vol. 115, no. 3, (2003-10-31), pages 281-292

P2: TEMPLE SALLY: "Embryonic stem cell self-renewal, analyzed." CELL, vol. 115, no. 3, (2003-10-31), pages 247-248

1.2. The present application validly claims priority from 16.10.2003. Any documents cited in the International Search Report as P documents have therefore not been considered as comprised in the prior art relevant for the present application.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. No meaningful examination could be performed for claims 1-30 partially, for the following reason:

1.1. Rule 66. 1.(e) (PCT):
No complete international search report has been established for said claims (see Form PCT/ISA/210). Accordingly, said claims need not be the subject of international preliminary examination.

2. Claims 23-27 -as far as they concern *in vivo* methods- relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. **Novelty and Inventive step** (Article 33(2) and (3) PCT)

1.1. The present application is related to WO03095628 based on the observation that BMP (an anti-neurogenesis factor in the early embryo) in combination with LIF (acting through gp130 receptor and activating STAT3) supports self-renewal of mouse embryonic stem (ES) cells in serum-free culture. In the present application it is concluded that the critical contribution of BMP is to induce expression of Id (Inhibitor of differentiation, negative regulator of bHLH transcription factors) genes via the Smad pathway. Exposure to fibronectin and serum also increases Id expression in mouse ES cells, which may explain the essential action of serum. Overexpression of Nanog (a homeodomain protein) maintains constitutive expression of Id. Forced expression of Id liberates ES cells from BMP or serum dependence and allows self-renewal in LIF alone. Applicant's attention is drawn to the fact that the subject-matter searched has been significantly restricted for the reasons given in Form PCT/ISA/210.

1.2. The searchable gist of the application (see Item III), i.e. the finding that Id is critical for the self-renewal of mouse ES cells, is neither disclosed in nor suggested by the available prior art. Said gist of claims 1-29 is thus found to be novel and inventive under the terms of Article 33(2) and (3) PCT.

1.3. New claim 30 is merely defined by the method it was obtained by. It is hereby noted that for a product to be considered novel, the product has to be novel *per se*, irrespective of the method it is obtainable by. It becomes evident that claim 30, relating to "a cell obtainable by a method" according to preceeding claims, cannot

possibly be considered novel or inventive under the terms of Article 33(2) and (3) PCT.

2. **Industrial applicability** (Article 33(4) PCT)
The subject-matter of the claims for which an opinion has been established (see Item III) appears to be industrially applicable under the terms of Article 33(4) PCT.

Re Item VIII

Certain observations on the international application

1. Although the gist of the present application appears to be novel, inventive and industrially applicable, the drafting of the present claims suffers major deficiencies as pointed out in the reasons for the restricted search on Form PCT/ISA/210. Several claims are found not to comply with the requirements of Articles 5 and 6 (PCT). Said claims refer to agents, agonists, and activators without giving a true technical characterisation of such compounds. Moreover no such compounds are disclosed in the present application. In consequence the scope of said claims is ambiguous and vague and their subject-matter is neither sufficiently disclosed nor supported. These deficiencies are so severe as to render a meaningful examination impossible.

2. Applicant's attention is drawn to the fact that, upon entry into the regional phase, patentability of claims relating to human embryos may underlie restrictions based on moral grounds. The EPO, for example, does not recognize as patentable the subject-matter of claims to the cloning of human beings, the modification of the germ line identity of human beings and the use of human embryos for industrial or commercial purposes (Article 53(a) and Rule 23d EPC).

CLAIMS:

1. Use of an Id gene product in promoting self-renewal of pluripotent cells in culture.

2. Use according to Claim 1 of a combination of the Id gene product with an activator of a gp130 downstream signalling pathway.

3. Use of a combination of
   (i) an agent that increases Id protein expression or activity; and
   (ii) an activator of a gp130 downstream signalling pathway,
   in promoting self-renewal of pluripotent cells in culture in medium that is free of serum and free of serum extract.

4. Use according to any of Claims 1-3, wherein the activator of a gp130 downstream signalling pathway is LIF.

5. Use according to any of Claims 1-4, wherein the pluripotent cells are embryonic stem cells.

6. Use according to Claim 5 wherein the embryonic stem cells are mouse cells or human cells.

7. Use according to any of Claims 3-6 wherein the agent (i) is selected from fibronectin, agonists of the fibronectin receptor, activators of integrin signalling, nanog, and homologues of all of the aforementioned that induce Id gene expression or Id protein activity.

8. Use according to any of Claims 1-7, comprising inducing expression of an Id gene.

9. Use according to any of Claims 1-8, comprising genetically manipulating a pluripotent cell so that it expresses an Id gene.

10. Use according to any of Claims 1-9, comprising introducing into a pluripotent cell a vector comprising an Id gene.

11. Use according to any of Claims 1-11 wherein the Id gene product is an Id protein.

12. A method of promoting self-renewal of a pluripotent cell in culture in medium that is free of serum and free of serum extract, comprising (1) expressing an Id gene or inducing expression of an Id gene in the cell, or culturing the cell in medium containing an Id protein, and (2) activating GP130 downstream signalling.

13. A method according to Claim 12, comprising expressing an Id gene episomally in the cell.

14. A method according to Claim 13 comprising expressing an Id gene from an episomal vector comprising an inducible promoter.

15. A method according to any of Claims 12-14, comprising stimulating gp130 downstream signalling by culturing the cell in medium comprising a cytokine acting through gp130.

16. A method according to Claim 15 wherein the cytokine is selected from LIF, CNTF, Cardiotrophin, Oncostatin M and a combination of IL-6 plus sIL-6 receptor.

17. Use of a combination of:-

(a) a direct activator or effector of Id gene expression and/or Id protein activity, other than one acting through a receptor of the TGF-β superfamily; and

(b) an activator of a gp130 downstream signalling pathway, in promoting self-renewal of a pluripotent cell in culture in medium that is free of serum and free of serum extract.

18. A method of culture of ES cells so as to promote ES cell self renewal in medium that is free of serum and free of serum extract, comprising maintaining the ES cells in medium containing:-

(a) an Id protein or a direct activator or effector of Id gene expression and/or Id protein activity, other than one acting through a receptor of the TGF-β superfamily; and

(b) an activator of a gp130 downstream signalling pathway.

19. A method of culture of ES cells, comprising:-

(a) maintaining the ES cells in a pluripotent state in culture, optionally on feeders, in the presence of a cytokine acting though gp130 and serum or an extract of serum;

(b) passaging the ES cells at least once;

(c) withdrawing the serum or the serum extract from the medium and withdrawing the feeders if present, so that the medium is free of feeders, serum and serum extract; and

(d) subsequently maintaining ES cells in a pluripotent state in the presence of:-

(i) a direct activator or effector of Id gene expression and/or Id protein activity, other than one acting through the receptor of the TGF-β superfamily; and

(ii) an activator of a gp130 downstream signalling pathway.

20. A method of obtaining a transfected population of ES cells, comprising:-

(a) transfecting ES cells with a construct encoding a selectable marker operably linked to a promoter that expresses the selectable marker preferentially in ES cell;

(b) plating the ES cells;

(c) culturing the ES cells in the presence of

(i) a direct activator or effector of Id gene expression and/or Id protein activity, other than one activator acting through a receptor of the TGF-β superfamily; and

(ii) an activator of a gp130 downstream signalling pathway; and

(d) selecting for cells that express the selectable marker.

21. A method of culture of ES cells in medium that is free of serum and free of serum extract, comprising transferring an individual ES cell to a culture vessel and culturing the ES cell in the presence of

(a) a direct activator or effector of Id gene expression and/or Id protein activity, other than one acting through a receptor of the TGF-β superfamily; and

(b) an activator of a gp130 downstream signalling pathway,

so as to obtain a clonal population of ES cells, all of which are progeny of a single ES cell.

22. A medium for self-renewal of ES cells, comprising:-

(1) basal medium;

(2) a direct activator or effector of Id gene expression and/or Id protein activity, other than one acting through a receptor of the TGF-β superfamily;

(3) an activator of gp130 downstream signalling pathways; and

(4) an iron transporter;

wherein the medium is free of serum or serum extract.

23. Use of an agent that increases Id protein activity in a pluripotent cell, in promoting self-renewal of the pluripotent cell in medium that is free of serum and free of serum extract.

24. Use according to Claim 23 wherein the agent increases the amount of Id protein in the cell.

25. Use according to Claim 23 wherein the agent comprises a composition comprising an Id protein and a translocation domain.

26. Use according to claim 25, wherein the composition comprises a fusion protein of the Id protein and the translocation domain.

27. Use according to claim 25, wherein the translocation domain comprises TAT, VP22 or a penetratin

28. A method of obtaining a pluripotent cell in medium that is free of serum and free of serum extract, comprising
expressing an Id gene or inducing expression of an Id gene in a cell, or culturing a cell in medium containing an Id protein, and activating gp130 downstream signalling in the cell, wherein the cell is obtained from somatic cells or tissue of a fetus or adult.

29. A method according to claim 28, wherein the pluripotent cell is characterised by being positive for Rex1, Oct4 and nanog.

30. A cell obtained by a method according to any of claims 28 to 29.